

The University of Chicago

A STUDY OF THE VEGETATIVE
PHASES OF EPHEDRA

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1933

By
PAUL DIRKS VOTH

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A STUDY OF THE VEGETATIVE PHASES OF EPHEDRA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 457

PAUL D. VOTH

(WITH TWENTY-FIVE FIGURES)

Introduction

According to STAPP (15), species of *Ephedra* are found in the drier, warmer regions of every continent except Australia. The genus has received, together with *Gnetum* and *Welwitschia*, much attention in phylogenetic studies. The value of certain Asiatic species of *Ephedra* as a source of ephedrine, and of other species growing in the arid southwestern part of the United States as browse for cattle, sheep, and goats (6), has increased the economic interest in the genus.

The germination of *Ephedra* has been described in some detail by STRASBURGER (17) and by BOWER (4). Both stress the fact that *Ephedra* differs from *Gnetum* and *Welwitschia* in that no lateral projection of the hypocotyl (foot) is present in the former. BOWER (4) postulates that the suspensor and the living remains of the nucellus may act as conductors of nutrient material from the megagametophytic tissue to the seedling. SCHACHT (14) as well as HILL and DE FRAINE (10) show *Ephedra* seedlings with a structure that appears to be a foot. MARKGRAF (12), although he illustrates some of the stages of germination after HILL and DE FRAINE, correctly pictures the seedling without a feeding organ.

STRASBURGER (17) was the first to describe the root-stem transition in the hypocotyl of *Ephedra*. He pointed out that the vascular bundles in the hypocotyl follow a course similar to that followed by the bundles in the hypocotyl of *Araucaria*. The only other description of the vascular system in the hypocotyl of *Ephedra* is given by HILL and DE FRAINE (10). In their illustration a semicircular girdle of transfusion tissue connects the two hypocotyledonary bundles at a distance considerably below the cotyledons. No such condition was found in the species under investigation.

Apparently no detailed studies have been made to determine the vascular situation in the epicotyl of an *Ephedra* seedling. The course of the vascular bundles in the stem tip and the larger branches of plants past the seedling stage has been the subject of investigation since the time of NÄGELI (13). His longitudinal diagram of the stem of *E. vulgaris* roughly agrees with the situation in the stem of *E. pedunculata*, the species upon which the greater part of the present paper is based. GEYLER (9) extended the observations of NÄGELI to *E. equisetiformis* Webb and Berth., to *E. helvetica* C. A. Meyer (*E. distachya* Gand.), and to *E. campylopoda* C. A. Meyer (*E. distachya* Durv.), all of which were in accord with the species described by NÄGELI. STRASBURGER (17, pp. 77-78) reported that *E. altissima* does not have the customary eight bundles in the stem but only six, owing to the non-divergence of a pair of bundles in the second internode below the node at which the bundles diverge into the leaves. The vascular bundles in the stem continue for two internodes before they diverge into the opposite leaves. DE BARY (1) reviewed the vascular condition in the different species of *Ephedra* and referred to the work of STRASBURGER (17) on *E. campylopoda*, who showed that a "complementary" bundle is differentiated parallel to and between the two foliar bundles in the internode just below the node at which the foliar bundles diverge into the leaves. The "complementary" bundle evidently is lost in the girdle of xylem which is found at every node. This explains the occurrence of ten bundles in the stem of this species.

The number of bundles in the stem of *Ephedra* has been made one of the diagnostic characteristics of a new Asiatic species described by STAPF (16). LIU (11) has since shown that the number (eight) mentioned by STAPF is not constant, and that as many as ten bundles are found in the older parts of the plant.

STRASBURGER (17) and BERTRAND (2) have compared the anatomical features of *Ephedra* and the other Gnetales with those of the Coniferales. EVANS (7) described the anatomical features of several North American species of *Ephedra*, emphasizing the xeromorphic structures of stem and leaf. More recently GEORGE (8) has concluded that anatomically *Ephedra* is gymnospermous in its affinities. COULTER and CHAMBERLAIN (5) have discussed some of the anatom-

ical features of the Gnetales in relation to phylogeny. BOODLE and WORSDELL (3) compared the anatomy of *Ephedra* with that of *Casuarina*.

Materials and methods

Seeds and vegetative portions of *Ephedra pedunculata* Englm. were collected about 8 miles southeast of Lubbock, Texas, in May and June, 1931 and 1932. The vegetative parts were preserved in a formalin-alcohol solution containing 6 per cent commercial formalin and 60 per cent of 95 per cent alcohol. The seeds with their surrounding red, fleshy involucre bracts were air dried and later germinated in the University of Chicago greenhouses.

Most phylogenetic studies on *Ephedra* have been made on plants old enough to have lost many of their primary features. This paper records some observations on the primary stelar condition of the seedling.

Stem tips of *E. viridis* Coville, also a North American species inhabiting the arid southwest, and of *E. allissima* Desf. (?), a North African species, were used for comparison.

Seedlings used for the study of the root-stem transition were 33 days old; those of the epicotyl, 51 days. Formalin-alcohol (6-60 per cent), Flemming's medium, or Sax's modification of Nawaschin's fluid were used as fixing agents. Serial sections were cut 12 μ thick.

Investigation

SEEDLING

Under favorable conditions the seeds germinate in about ten days. The hypocotyl elongates rapidly and in two to five days the primary root, devoid of laterals, has grown two or three inches. Concurrently the cotyledons elongate, especially in their basal portions. The seed coat either remains in the substratum and the two cotyledons (bent as a knee in their basal portion) emerge completely, or the seed coat is brought above ground by the cotyledons, their tips still in position to absorb food from the remaining tissue of the megagametophyte. After a short period of growth the upper part of the hypocotyl straightens out. In the greenhouse, in direct light, the cotyledons average 5 cm. in length and 1 mm. in diameter. They are linear in

outline and in cross-section are roughly semicircular, their flattened sides facing each other and the stem. The cotyledons function for a time as photosynthetic organs.

At about 15 mm. below the divergence of the cotyledons the axis is definitely recognizable as primary root. This portion of the axis is smaller in diameter than the hypocotyl. The cortex, which is soon crushed, becomes darkened.

The diameter of the axis is much greater at the level of the divergence of the cotyledons, the bases of which form a cotyledonary sheath about 1.5 mm. in length.

The leaves are opposite, in decussate arrangement. Near the growing point the internodes are very short, but when mature they average 3 cm. in length. During the early life of the seedling, when the primary root continues to grow and the lateral roots are formed, growth of the epicotyl is very slow; later the rate of elongation is largely dependent upon water supply.

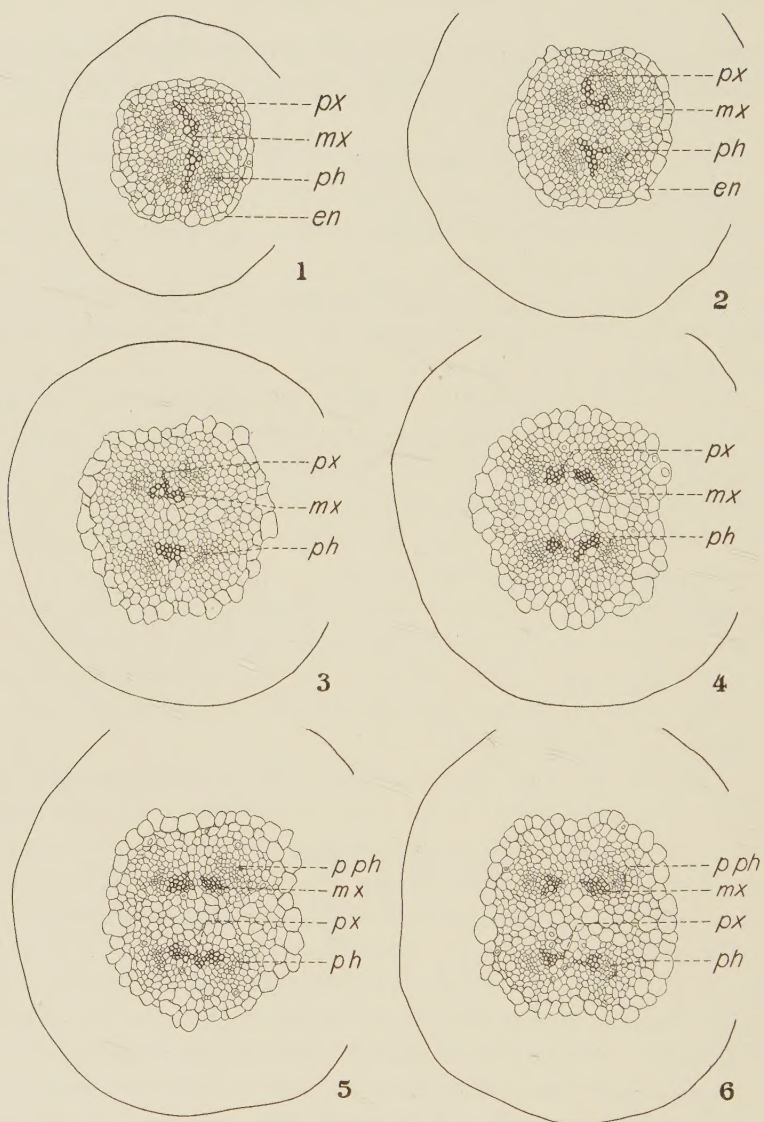
In the greenhouse the leaves of *Ephedra* remain functional for more than a year, but in the arid native habitat they become dry and scalelike within a few weeks. The stem functions as a photosynthetic organ until secondary growth and weathering disrupts the palisade layers in the cortex.

As will be seen from figures 21 and 22, buds can form in the axils of the cotyledons. Such buds, as well as those in the axils of the leaves, become active when the stem tip above them is clipped or grazed off. More than one bud may form in an axil, which produces the whorled appearance of branches of the older native plants.

PRIMARY ROOT AND ROOT-STEM TRANSITION

The primary root is diarch, exarch. In some roots (fig. 1) a few cells in the center of the axis remain parenchymatous, in others the protostelic condition is attained only in the more distal portions.

In seedlings a month old the protoxylem elements are spiral or modifications of this general type. At places bordered pits occur on these elements. These pits are more numerous on walls where two tracheids are adjoined, but they also occur on walls which border on parenchyma cells. The walls of the metaxylem tracheids which are differentiated first (abaxial) are reticulate, while those differentiated



FIGS. 1-6.—Transverse sections of primary root and hypocotyl of a seedling 33 days old (only stele and endodermis shown in detail): Fig. 1, cross-section through primary root just below hypocotyl region, showing four phloem groups (*px*, protoxylem; *mx*, metaxylem; *ph*, phloem; *en*, endodermis). Figs. 2, 3, cross-sections through lower part of hypocotyl. Metaxylem is being differentiated abaxially. Figs. 4-6, cross-sections of hypocotyl showing tangential divergence of each primary xylem group (*p ph*, proto-phloem). $\times 47$.

later are more heavily thickened and possess bordered pits with slit openings and tertiary thickenings. The innermost metaxylem tracheids do not mature until secondary growth has begun. At no level are more than four protoxylem elements visible at a protoxylem point.

The phloem of the primary root is difficult to distinguish. Several millimeters below the hypocotyl two phloem groups alternating with the two xylem groups are visible in cross-section. Near the hypocotyl four groups of elongated cells of small diameter, each with an elongated nucleus, are discernible lateral to the primary xylem groups (fig. 1); hence each xylem strand is flanked on either side by a phloem strand. No crushed protophloem is recognizable in the primary root.

A pericycle three or four cells in width, opposite the xylem groups and wider over the phloem, forms the periphery of the stele. At places this pericyclic zone of cells is ill-defined and merges into the parenchymatous rays which are at right angles to the length of the xylem groups.

At a level of 2 cm. below the divergence of the cotyledons (the primary root), the Casparian strips on the radial walls of the cells are so small that they are scarcely visible. At a higher level, in the hypocotyl, the Casparian strips are pronounced but are entirely unrecognizable at a level about 1 cm. below the divergence of the cotyledons. In general the endodermal cells are larger than the cells of the stele adjoining them, and are devoid of intercellular spaces.

The cortex of the root is usually less than six or seven cells in width. The cells are spherical with relatively small intercellular spaces. The epidermis of somewhat elongated tabular cells is soon sloughed off. Root hairs are found only rarely. With rapid growth of the root the endodermis often collapses early and the entire cortex sloughs off. No evidence of a periderm was found.

As indicated in figure 2, which is a cross-section 2 mm. higher than figure 1, the axis increases slightly in diameter. Viewed at this level the wedges of primary xylem appear more obtuse and farther apart. In reality the protoxylem points remain in the same position relative to the diameter of the axis, while cells more lateral and more abaxial to them become differentiated and mature into metaxylem.

At the same time a larger number of cells in the center of the axis remain parenchymatous and constitute the pith. At this level (fig. 2; also fig. 17, level 2') and at all succeeding levels the axis is a dissected siphonostele.

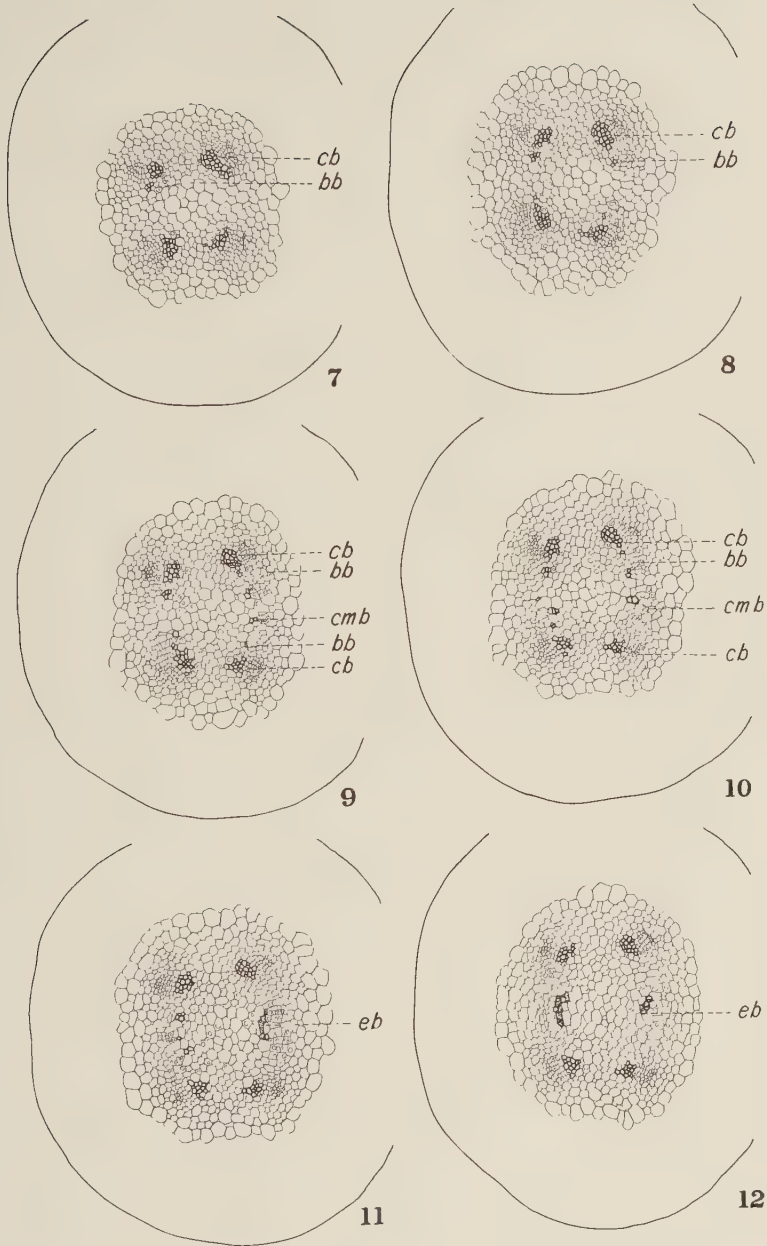
As shown in figure 3 (also fig. 17, level 3'), the metaxylem continues to be differentiated more centrifugally and tangentially higher in the hypocotyl, so that in cross-section each primary xylem group has the shape of an equilateral triangle with the protoxylem point exarch.

About 3.5 mm. below the divergence of the cotyledons (fig. 4), a section of the hypocotyl shows that each of the two exarch primary xylem strands becomes divided tangentially, appearing as a pair of xylem groups. These four strands of primary xylem continue into the cotyledons although they become endarch in arrangement; and each pair constitutes, together with their associated phloem groups, a cotyledonary trace. Casparian strips are best developed at this level. In all succeeding cross-sections the endodermis is doubtfully recognizable by its size, relative position, and the lack of intercellular spaces.

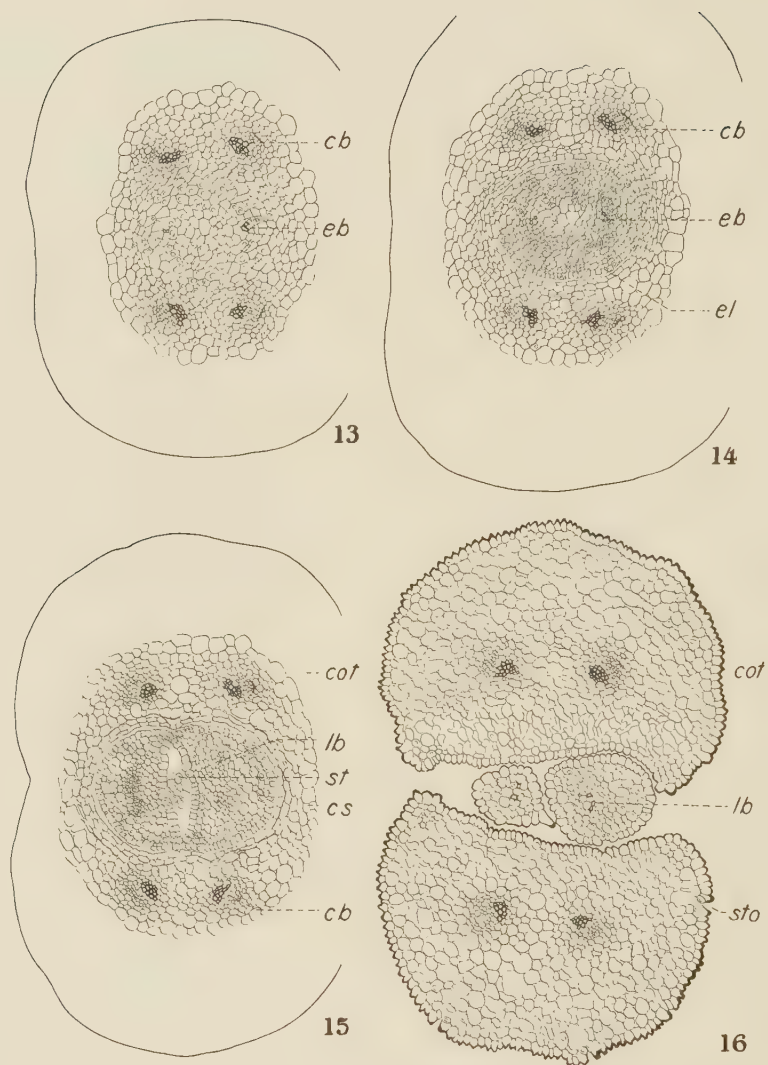
Cross-sections nearer the cotyledons show the following:

1. The metaxylem elements are differentiated in a progressively abaxial direction with reference to the protoxylem. At a level indicated by figure 7 (and fig. 17, level 7') a condition exactly intermediate between exarch and endarch is shown. In succeeding higher levels the centrifugal differentiation continues, so that when the cotyledonary trace enters the cotyledons the bundles are completely endarch.

2. At the level of cotyledon divergence each cotyledonary bundle gives rise to a branch bundle (fig. 7 and following figures). The branch bundles depart adaxially and slightly toward the outside of the axis, so that at a slightly higher level a pair from opposite cotyledons meet. Just before the bundles of each pair anastomose with one another, another bundle composed of a few tracheids is differentiated between them. This new bundle (figs. 9, 10, *cm b*; fig. 17, levels 9', 10'), which is designated as a complementary bundle, arises *de novo* with no connection with other vascular tissue at a lower level. This short bundle anastomoses with the converging



FIGS. 7-12.—Transverse sections of hypocotyl of a seedling 33 days old: Figs. 7, 8, appearance of branch bundles (*bb*) from cotyledonary bundles (*cb*). Figs. 9, 10, appearance of complementary bundle (*cm b*) with no vascular connection at lower end. (In fig. 10 the lower right branch bundle has failed to mature at this level.) Figs. 11, 12, anastomosis of a pair of branch bundles with each other and with complementary bundle to form epicotyledonary bundle (*eb*). $\times 47$.



FIGS. 13-16.—Transverse sections of epicotyl (*el*) and cotyledons of a seedling 33 days old: Fig. 13, divergence of abaxial surface of cotyledons (*cb*, cotyledonary bundle; *eb*, epicotyledonary bundle). Fig. 14, divergence of adaxial surface of cotyledons. Fig. 15, transection through tip of axis. Cotyledons (*cot*) have not diverged from each other at this level (*lb*, leaf bundle; *st*, stem tip; *cs*, cotyledonary sheath). Fig. 16, transverse section through cotyledons and tips of first foliage leaves (*sto*, stoma). $\times 47$.

pair of branch bundles from opposite cotyledonary bundles to form a single epicotyledonary bundle (figs. 11, 12, *eb*). Each epicotyledonary bundle is therefore connected at its base to three bundles; two

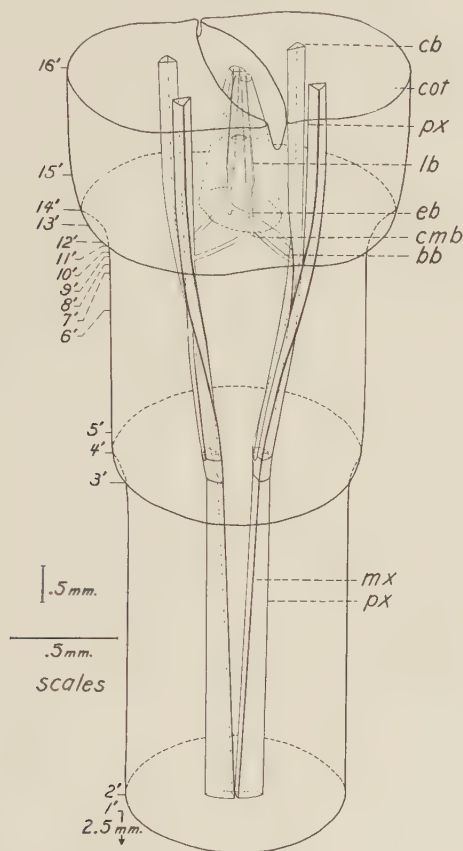


FIG. 17.—Schematic, longitudinal diagram of hypocotyl showing transition from root to cotyledons (drawn to scale with the levels of figs. 1–16 indicated as 1' to 16'). Horizontal and vertical proportions are respectively three and one (*cb*, cotyledonary bundle; *px*, protoxylem; *mx*, metaxylem; *lb*, leaf bundle; *eb*, epicotyledonary bundle; *cm b*, complementary bundle; *bb*, branch bundle). $\times 47$.

of these are tangential extensions from opposite cotyledonary bundles, and one is a free bundle, arising *de novo* between these two.

At a later age the epicotyledonary bundle becomes a wide crescent

tic girdle of vascular tissue resulting from the maturation of additional metaxylem elements. From the two crescentic girdles of vascular tissue at each node (in the first node designated as epicotyledonary bundles) two pairs of bundles arise, while two pairs continue through this girdle into the next internode and depart into the succeeding higher leaves (figs. 18-25).

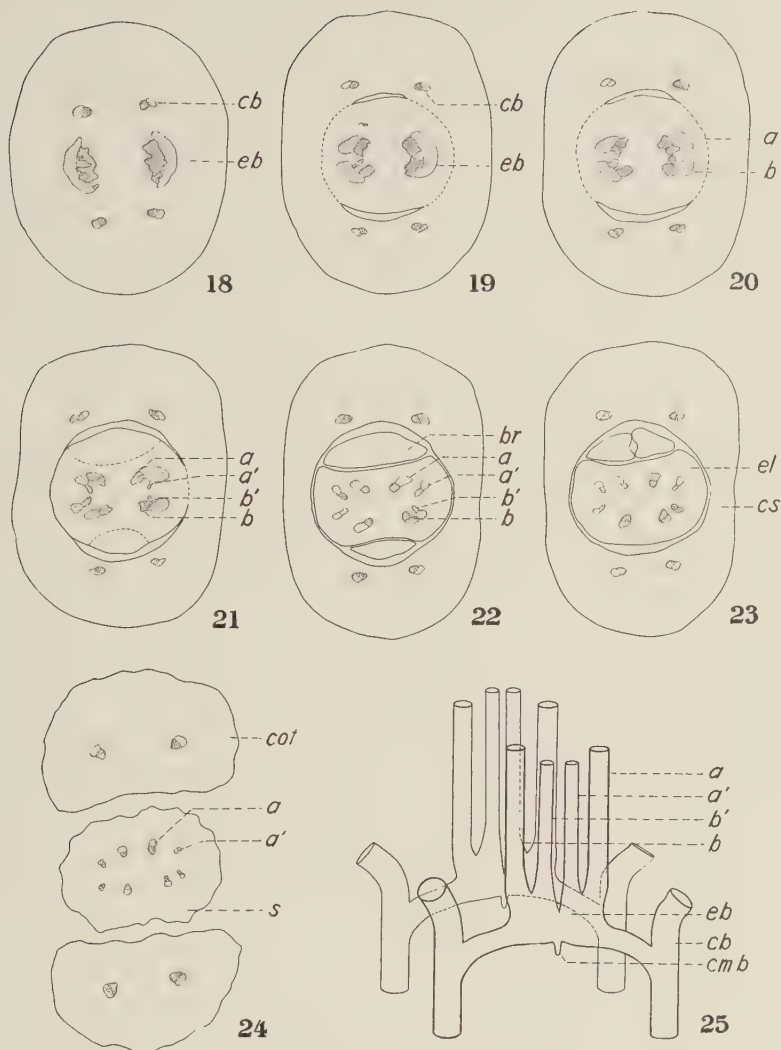
COTYLEDONS

The opposite cotyledons as well as the opposite leaves do not diverge from each other for some distance above the node, thus forming a sheath (figs. 14, 15). The flattened side of the semicircular cotyledons (as seen in cross-section) usually is concave, conforming to the curvature of the axis. The epidermis of elongated cells (four to five times as long as broad) is heavily cuticularized. In cross-section the radial and tangential dimensions of the cells are approximately the same. The stomata are sunken, the aperture communicating with the outside being guarded by four to six epidermal cells (7). Between the epidermis and the two vascular bundles of each cotyledon chlorenchymatous cells of various shapes are found. Those near the bundles are compact while those near the periphery have air spaces between them. Under each stoma the air space is relatively large. Near the base of the cotyledon the adaxial subepidermal cells are elongated palisade cells, with their long axis perpendicular to the axis of the foliage organ. In the upper end of the cotyledon (which emerged last from the seed coat) no definite palisade layer is recognizable. In the greenhouse the cotyledons function as photosynthetic organs for more than six months.

The phloem, which in the primary root was in four groups, two lateral to each of the two xylem groups, is differentiated abaxially at successively higher levels, so that in the cotyledons it is completely centrifugal with reference to the xylem. In the cotyledons the transition is complete; thus only the hypocotyl and the cotyledons have been directly involved.

EPICOTYL AND STEM

At the age of 33 days only two decussate pairs of leaves have formed. The lower internode of these two shows some provascular



FIGS. 18-25.—Figs. 18-24, transverse sections at level of cotyledon divergence and through epicotyl of a seedling 51 days old. Figs. 18, 19, transverse sections at cotyledonary node. In fig. 19 the adaxial surface of the cotyledons is diverging from the axis and each epicotyledonary bundle (*eb*) is beginning to diverge at right angles to its length (*cb*, cotyledonary bundle). Figs. 20, 21, each epicotyledonary bundle (crescentic girdle) diverges equally to give rise to two bundles, *a* and *b*, which in turn diverge into *a*, *a'*, and *b'*, *b*. Fig. 22, cotyledons diverged completely from epicotyl. Buds in axils of cotyledons have given rise to branches (*br*). Figs. 23, 24, eight bundles in the stem differentiated so that they are nearly equidistant from one another (*el*, epicotyl; *cs*, cotyledonary sheath; *col*, cotyledon; *s*, stem). $\times 25$.

Fig. 25.—Schematic diagram of vascular system in epicotyl and stem based on transverse sections shown as figs. 18 to 24 (*a*, *a'*, *b'*, *b*, as in figs. 21 and 22; *cm b*, complementary bundle). $\times 53$.

tissue, the upper one none. No cell elongation has taken place. In the lower internode two opposite points, alternate in arrangement with the cotyledons, soon are sufficiently mature to be identified as protoxylem and protophloem elements and are continuous with the epicotyledonary bundle. At a higher level each one of these points branches equally, each pair of bundles constituting a leaf trace. In figure 16 the xylem elements in the first pair of leaves above the cotyledons are more mature than those in figure 15. Growth by cell division and cell enlargement brings the foliar bundles farther apart in the more distal portions of the leaf.

In cotyledons and leaves which are fully grown, the two foliar bundles run nearly parallel to each other and extend to within two or three cells from the epidermis of the tip. The few cells which intervene between the ends of these bundles later mature into short cells with reticulate thickenings, that is, transfusion tissue.

In seedlings which are 51 days old secondary growth has begun in the root and hypocotyl, but practically no cambium has formed in the axis above the cotyledons. Beginning with a section through the cotyledonary node comparable to figure 12, the primary tissues have matured more completely (fig. 18). Instead of the two small protoxylem points (fig. 13), two opposite crescentic girdles of primary xylem subtended by two collateral phloem groups are present. These girdles alternate in arrangement with the cotyledons and are comparable to the cotyledonary plate in other plants. The cotyledonary bundles have diverged at a lower level, as described in connection with the transition.

As the adaxial surface of the cotyledon departs from the axis, each cotyledonary girdle branches equally in the direction of its longest dimension. The direction of this divergence is alternate with that of the cotyledonary trace (fig. 19). Following so closely as to be almost concurrent, each of the four bundles just formed diverges in a plane parallel to the first divergence. Although not all of the eight bundles thus formed depart at the same level, a slightly higher section (fig. 20) shows that eight distinct bundles are present in the axis. In subsequent sections the bundles are differentiated in such a position that they are nearly equidistant from one another. The

two pairs of bundles which departed last are slightly smaller and roughly form the corners of a parallelogram which has its longest dimension at right angles to the cotyledons. These two pairs of bundles constitute the leaf traces for the succeeding node (fig. 25).

Stem tips of *Ephedra pedunculata* and of *E. viridis* were compared to determine the vascular condition of the node. Beginning at a level represented in figure 25, the pair of bundles at the narrow end of the parallelogram diverge into the leaves just before the node is attained. At the node the remaining six bundles are anastomosed into two semicircular girdles, much as the six bundles constitute the girdles (cotyledonary plate) in the first node. The crescentic girdles alternate in position with those of the preceding and succeeding nodes. From the girdles at each node eight bundles arise as described for the cotyledonary node (figs. 18-25). Such origin explains the presence of eight bundles in the stem of *Ephedra* as described by earlier investigators (1, 9, 10, 13, 15, 17). Variations from this number can be explained on the basis of the non-divergence of two pairs of bundles and by the differentiation of a "complementary" bundle between the bundles which form the leaf trace for the succeeding node (17).

Ephedra viridis possesses eight distinct bundles in its stem but the lateral differentiation of metaxylem proceeds until a continuous cylinder of xylem is formed, making it appear as though secondary growth began very early in the stem. *Ephedra altissima* (?) resembles *E. pedunculata* in the appearance of the internodal bundles.

Summary

1. The root-stem transition is described for *Ephedra pedunculata* Englm. and the vascular condition of the stem is compared with that of *E. viridis* Coville and *E. altissima* Desf.

2. The primary xylem in the root of *Ephedra* is diarch, exarch. The two groups of primary phloem alternate with the xylem.

3. The transition occurs in the hypocotyl. Each primary xylem group diverges into two lateral branches both of which continue into the cotyledon. Differentiation and maturation of the xylem elements in each cotyledonary bundle proceed in such a manner that

at successively higher levels the metaxylem becomes constantly more abaxial. The cotyledonary bundles are exarch when they depart into the cotyledons.

4. At the first node the cotyledonary bundles each give rise to a branch bundle. These bundles are differentiated toward each other in pairs. When these converging branch bundles anastomose with each other a third but shorter bundle joins them. The latter bundle arises *de novo*.

5. In an older stem the differentiation of additional metaxylem elements to the three bundles, forming an epicotyledonary bundle, gives rise to a crescentic girdle with its longest axis parallel to the leaves diverging at that node. From the pair of girdles at each node eight bundles arise and continue separately through the succeeding internode. Above the second node each bundle continues for two internodes before it departs into a leaf.

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LITERATURE CITED

1. DE BARY, ANTON, Comparative anatomy of the vegetative organs of the phanerogams and ferns. English transl. London. 1884.
2. BERTRAND, C. E., Anatomie comparée des tiges et des feuilles chez les Gnétacées et les Conifères. Ann. Sci. Nat. Bot. 5^e Sér. 20:5-153. 1874.
3. BOODLE, L. A., and WORSDELL, W. C., On the comparative anatomy of the Casuarineae, with special reference to the Gnetaceae and Cupuliferae. Ann. Bot. 8:231-264. 1894.
4. BOWER, F. O., The germination and embryogeny of *Gnetum Gnemon*. Quar. Jour. Micro. Sci. n.s. 22:278-298. 1882.
5. COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of gymnosperms. Chicago. 1917.
6. DAYTON, W. A., Important western browse plants. U.S. Dept. Agric. Misc. Publ. 101. 1931.
7. EVANS, W. H., The stem of *Ephedra*. BOT. GAZ. 13:265-268. 1888.
8. GEORGE, LUCIENNE, Sur l'anatomie de l'*Ephedra altissima* Desf. Compt. Rend. Assoc. Franc. l'avan. Sci. 54:216-219. 1930.
9. GEYLER, H. T., Ueber den Gefässbündelverlauf in den Laubblattregionen der Coniferen. Jahrb. Wiss. Bot. 6:55-208. 1867.
10. HILL, T. G., and DE FRAINE, E., On the seedling structure of gymnosperms. IV. Ann. Bot. 24:319-333. 1910.

11. LIU, J. C., The number of vascular bundles in *Ephedra sinica* and *E. equisetina*. Lingnan Sci. Jour. 7:167-176. 1929.
12. MARKGRAF, FR., in ENGLER, A., and PRANTL, K., Die natürlichen Pflanzenfamilien. 13:407-441. 1926.
13. NÄGELI, CARL, Beiträge zur wissenschaftlichen Botanik. Heft 1. Leipzig. 1858.
14. SCHACHT, HERMANN, Lehrbuch der Anatomie und Physiologie. 2 vols. Berlin. 1856.
15. STAPF, OTTO, Die Arten der Gattung *Ephedra*. Denksch. K. Akad. Wiss. (Wien) Math.-Nat. 56:(2) 1-112. 1889.
16. ———, Ma Huang of Chili (*Ephedra sinica* Stapf). Kew Bull. Misc. Inform. 1927:133-134. 1927.
17. STRASBURGER, EDUARD, Die Coniferen und die Gnetaceen. Jena. 1872.

